PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P208746PCT1 P27609PC01	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/NL2004/000428	International filing date *(day/month/year)* 16.06.2004	Priority date *(day/month/year)* 17.06.2003
International Patent Classification (IPC) or national classification and IPC C12Q1/68, C12N15/11		
Applicant KEYGENE N.V. et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 5 sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
07.01.2005	07.06.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Hillenbrand, G Telephone No. +49 89 2399-8428

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/NL2004/000428

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

   Description, Pages

   1-75 as originally filed

   Claims, Numbers

   1-30 received on 15.03.2005 with letter of 15.03.2005

   Drawings, Sheets

   1-7 as originally filed

   ☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-30
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-30
Industrial applicability (IA)	Yes:	Claims	1-30
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed

   ☐ filed together with the international application in computer readable form

   ☒ furnished subsequently to this Authority for the purposes of search and/or examination

   ☒ received by this Authority as an amendment on

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

D1: US-A-5 424 413 (HOGAN JAMES J ET AL) 13 June 1995 (1995-06-13)

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Novelty (Article 33.2 PC) and Inventive step (Article 33.3 PCT)

The subject-matter of claims 1-30 is not considered novel over **D1,** cited as nearest prior art (Article 33(2) PCT).

The applicant has incorporated the content of claim 3 into claim 1 in order to establish novelty over **D1**. The facultative functional feature that the first and second target sections are capable of being ligated to each other when hybridized to S1 and S2 does, however, not establish novelty over **D1**. As can be derived from Fig. 4-5 and claim 36 of **D1**, the two separate target specific regions that hybridize to a target nucleic acid sequence **are covalently joined to each other** and thus comply with the above mentioned facultative functional requirement. Thus, we maintain our position that **D1** is novelty destroying for the subject-matter of claims 1-30. **D1** disloses already nucleic acid hybridization probes having at least one nucleic acid strand which has at least two separate target specific regions that hybridize to a target nucleic acid sequence (which can be covalently joined to each other), and at least two distinct arm regions that do not hybridize with the target nucleic acid but possess complementary regions that are capable of hybridizing with one another. These regions are designed such that, under appropriate hybridization conditions, the complementary arm regions will not hybridize to one another in the absence of the target nucleic acid; but, in the presence of target nucleic acid the target-specific regions of the probe will anneal to the target nucleic acid, and the complementary arm regions will anneal to one another, thereby forming a branched nucleic acid structure. Thus, at present it is not visible which defined technical feature(s) could distinguish the pair of oligonucleotide probes/kits as claimed in claims 1-19 and 29-30 and methods of claims 20-27 from the probes/kits and methods described already in **D1**.

Even if the applicant would establish formal novelty over **D1** (by the incorporation of **true technical features of the claimed pair of oligonucleotides** into claim 1), it is at present not visible which surprising/advantageous properties of the claimed matter could involve an inventive step over the teachings of **D1** (Article 33.3 PCT).

Amended Claims

1. A pair of oligonucleotide probes (K) comprising: (68)

a) a first oligonucleotide probe (P1) that comprises a first clamp section (C1), that is capable of hybridising to a second clamp section (C2) of a second oligonucleotide probe (P2), and a first target section (T1) that is capable of hybridising to a first section (S1) of a target DNA sequence (D) to be detected;

b) a second oligonucleotide probe (P2) that comprises a second clamp section (C2), that is capable of hybridising to the first clamp section (C1) of the first oligonucleotide probe (P1), and a second target section (T2) that is capable of hybridising to a second section (S2) of the target DNA sequence (D) to be detected; ρ

2. A pair of oligonucleotide probes according to claim 1, wherein the first and second target sections (S1, S2) are located preferably adjacent to each other on the target DNA sequence (D).

3. ~~A pair of oligonucleotide probes according to claim 1 or 2~~ wherein the first and second target sections (T1, T2) are capable of being ligated to each other when hybridised to S1 and S2.

4. A pair of oligonucleotide probes according to claim 1, wherein the clamp sections (C1, C2) have melting temperature Tm_c which is higher than the melting temperature Tm_t of each of the target sections (T1, T2).

5. A pair of oligonucleotide probes according to claim 4, wherein the Tm_c of the clamp sections C1/C2 is at least 1 °C, preferably 5 °C more preferably 10 °C higher than the highest Tm_t of the two target sections T1 and T2.

6. A pair of oligonucleotide probes according to claim 1-5 wherein the GC content of clamp section ranges from more than 50 to 100%, preferably more than 60%, more preferably more than 70%, most preferably more than 80 % and is preferably in the range of 90-100%.

6
~~7.~~ A pair of oligonucleotide probes according to claim 1-~~6~~5, wherein the clamp section comprises, at least one, preferably at least one, more preferably at least 2, 3, 4 ,5 nucleotides selected from the group consisting of G's and C's, more than each of the target sections T1 or T2 of comparable length.

7
~~8.~~ A pair of oligonucleotide probes according to claim 1-~~7~~4, wherein the clamp sections C1 and/or C2 comprises nucleotides that have an increased binding affinity compared to conventional nucleotides.

8
~~9.~~ A pair of oligonucleotide probes according to claim 1-~~8~~7, wherein the clamp section comprises from 10 to 30, preferably from 15 to 25, more preferably from 18 to 24 nucleotides.

9
~~10.~~ A pair of oligonucleotide probes according to claim ~~9~~8, wherein the target sections each independently comprise from 15 to 30 preferably from 20 to 25 nucleotides.

10
~~11.~~ A pair of oligonucleotide probes according to claim 1-~~10~~9, wherein at least one of the oligonucleotide probes contains at least one primer binding site (B1, B2).

11
~~12.~~ A pair of oligonucleotide probes according to claim 1-~~10~~9, wherein the oligonucleotide probes contains at least one stuffer sequence (R1, R2).

12
~~13.~~ A pair of oligonucleotide probes according to claim 1-~~10~~9, wherein the target section (T1, T2) contains at least one allele-specific nucleotide.

13
~~14.~~ A pair of oligonucleotide probes according to claim ~~13~~12, wherein the allele-specific nucleotide is located at the end of a target section of the pair of probes.

14
~~15.~~ A pair of oligonucleotides according to claim ~~13~~12 or ~~14~~13, wherein least one additional probe (P3) is provided containing a target section (T3) that contains a

further allele specific nucleotide and wherein the probe (P3) be distinguished from P1 and/or P2 .

~~16~~ 15. A pair of oligonucleotides probes according to any of the preceding claims, wherein the first or the second probe comprises a further region that is not capable of annealing to the target nucleic acid sequence, which further region is located at the end of the first or second probe at the position of the junction site between the first and second sections of the target nucleic acid sequence.

~~17~~ 16. A pair of oligonucleotides probes according to claim ~~16~~ 15, wherein the further region is capable of creating a cleavage structure and whereby exposing the cleavage structure to a cleavage agent will result in cleavage of the cleavage structure when the cleavage structure and cleavage agent are incubated under conditions wherein cleavage can occur.

~~18~~ 17. A group comprising a least two pairs of probes according to any of the claims 1-~~17~~ 16.

~~19~~ 18. A group according to claim ~~18~~ 17, wherein the clamp sections C1 and C2 for each pair of probes are designed such that for each pair the combination of C1 an C2 forms a unique combination within the group such that each probe under given circumstances will selectively hybridise to one other probe in the group.

~~20~~ 19. Group according to claim ~~19~~ 18, wherein C1 and C2 further contain a unique sequence.

~~21~~ 20. Method for the detection of a target nucleotide sequence (D) in a sample comprising the steps of:

- providing a pair of oligonucleotide probes (K) as defined in any one of claims 1-~~17~~ 16 to the sample;
- allowing the probes to hybridise to the target sequence;
- optionally, providing a cleavage agent and cleaving any cleavage structure;
- ligating T1 and T2 when located adjacently on the target sequence (D); and

- detecting the presence or absence of any ligation products.

21 ~~22.~~ Method according to claim ~~21~~ 20, wherein the ligated probes are amplified prior to detecting.

22 ~~23.~~ Method according to claim ~~21~~ 20, wherein the target sequence is amplified prior to hybridisation of the probes.

20 22 ~~24.~~ Method according to claim ~~21-23~~, wherein more than one target nucleotide sequence is present (D1...Dn) in the sample to be analyzed and wherein more than one pair oligonucleotide probes (K1...Kn) are provided, corresponding to D1...Dn.

24 ~~25.~~ Method according to claim ~~21~~ 20 wherein the clamp section C1/C2 of each pair of oligonucleotide probes (K1...Kn) contains a unique sequence as defined in claim 19.

25 ~~26.~~ Method according to any of the preceding claims wherein the probes contain a unique sequence.

26 ~~27.~~ Method according to any of the preceding claims wherein detection is based on length, sequence and/or mass.

27 ~~28.~~ Method according to any of the preceding claims wherein the target sequence is selected from the group of DNA, RNA, polyA$^+$ RNA, cDNA, genomic DNA, organellar DNA such as mitochondrial or chloroplast DNA, synthetic nucleic acids, DNA libraries, clone banks or any selection or combinations thereof.

28 ~~29.~~ Set of at least three oligonucleotides suitable for SNP genotyping, comprising:

a) a first oligonucleotide probe (P1) that comprises a first clamp section (C1) that is capable of hybridising to a second clamp section (C2) of a second oligonucleotide

probe (P2) and a first target section (T1) that is capable of hybridising to a first section (S1) of a target DNA sequence (D) to be detected;

b) a second oligonucleotide probe (P2) that comprises a second clamp section (C2) that is capable of hybridising to the first clamp section (C1) of the first oligonucleotide probe (P1) and a second target section (T2) that is capable of hybridising to a second section (S2) of the target DNA sequence (D) to be detected;

c) at least a third oligonucleotide probe (P3) that comprises the second clamp section (C2) that is capable of hybridising to the first clamp section (C1) of the first oligonucleotide probe (P1) and the second target section (T2) that is capable of hybridising to the second section (S2) of the target DNA sequence (D) to be detected;

wherein the second probe and the third probe contain an allele-specific nucleotide , preferably located at the end of a target section of the set of probes;

wherein the allele-specific nucleotide of the second and the third probes corresponds to the alleles of the SNP to be detected;

wherein the second and the third probes contains a further (stuffer) section that discriminates between the (amplified) ligation products of the first probe with the second probe and the third probe.

29 ~~30~~ Kit comprising at least one pair of probes as defined in any of the claims 1-~~17~~ 16.

30 ~~31~~. Kit comprising at least one group of probes as defined in any of the claims ~~18~~-20 17-19.